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Phytochrome Control of the Expression of Two Nuclear Genes Encoding Chloroplast Proteins in *Lemna gibba* L. G-3[1]

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ABSTRACT

Hybridization probes for two nuclear-coded chloroplast proteins of *Lemna gibba* L. G-3 have been constructed in order to investigate phytochrome regulation of specific sequences. The first probe is a cDNA clone encoding the small subunit of ribulose 1,5-bisphosphate carboxylase. This probe was isolated from a set of *Lemna* cDNA clones in the bacterial plasmid pBR322. The second probe is a subclone of a genomic clone encoding the light-harvesting chlorophyll *a/b*-protein. This clone was isolated from a set of genomic clones constructed in the lambda vector Charon 4 with *L. gibba* DNA fragments generated by partial EcoR1 digestion. The identity of these clones was confirmed by *in vitro* translation of RNA which hybridized to the cloned DNA. Plants grown under continuous white light contain high concentrations of both RNA sequences; however, when these plants are put into darkness the concentration of these RNAs decreases rapidly relative to the total amount of RNA. Plants grown in the dark with intermittent red light (2 minutes/8 hours) and put into complete darkness for 8 days also contain lower concentrations of the sequences in the total RNA. One minute of red light after this dark period results in a rapid increase in the levels of RNA hybridizing to the probes. The effect of red light can be reversed by far-red light. These experiments demonstrate that phytochrome action can rapidly influence either the rates of transcription or the rates of degradation of these mRNAs.

The expression of two nuclear genes encoding chloroplast proteins is under the control of light in *Lemna gibba* (55, 56, 59). These proteins are the SSU[3] of RuBP carboxylase (17) and the light-harvesting Chl *a/b*-protein of the thylakoid membrane (52). Light affects the expression of these genes in *Lemna* by causing an increase in the amounts of the translatable mRNA encoding these polypeptides. The changes are accompanied by corresponding changes in the synthesis of the proteins *in vivo* (55, 59). This effect of light in *Lemna* is mediated by phytochrome (56). Correlated changes in translatable mRNA levels and the rates of *in vivo* synthesis of several polypeptides in response to light have also been reported in various other species of higher plants: these include a 32,000 D chloroplast membrane protein of *Spirodela* (35), maize (5), and mustard (31); phenylalanine ammonia lyase in cultured parsley cells (43); RuBP carboxylase in peas (6); phosphoenolpyruvate carboxylase in maize leaves (46); the Chl *a/b*-protein and NADPH-Pchlide-oxidoreductase in barley (1, 41). Apel (1, 2) has shown that in etiolated barley the response of the two mRNAs to light is mediated by phytochrome. However, none of these studies unravel exactly which step(s) in the expression of these genes are affected by phytochrome. Phytochrome action might affect the transcription of specific mRNAs or posttranscriptional processing, packaging, or degradation of these mRNAs. For example, it has been suggested (18) that red light acts by mobilizing preexisting mRNA onto polysomes. The studies discussed above could not have detected mRNA sequestered in an untranslatable form. To study such questions, we have isolated molecular hybridization probes that can be used for the quantitation of the concentration of specific RNA sequences, coding for the SSU of RuBP carboxylase and the Chl *a/b*-protein.

Both the SSU and the Chl *a/b*-protein are encoded by nuclear genes (11, 27, 29) and synthesized from polyadenylated (poly[A]) RNA on cytoplasmic ribosomes as precursor polypeptides which are transported into the chloroplasts (3, 12, 13, 16, 21, 55). In *Lemna*, it is not clear whether the two major polypeptides associated with the light-harvesting Chl *a/b*-complex (57) represent products of one or two genes, since antibodies against the two polypeptides immunoprecipitate a single band of 32,000 D from *in vitro* translation products (58).

We have used poly(A) RNA from light-grown plants to prepare a cDNA library from which we have isolated the sequence for the SSU of RuBP carboxylase. We have isolated a hybridization probe for the Chl *a/b*-protein RNA from a library of genomic DNA of *L. gibba* cloned in the Charon 4 derivative of bacteriophage lambda. We can now demonstrate that the content of these sequences is rapidly regulated by phytochrome action in *L. gibba* and we suggest that this regulation is occurring at the level of transcription.

MATERIALS AND METHODS

Growth of Plants. *Lemna gibba* L. G-3 was cultured aseptically in E medium with 1% sucrose in a growth chamber under constant white light at 27°C ± 2°C. Plants were also cultured in the dark with intermittent red light (2 min/8 h) in the same medium supplemented with 3 μM kinetin at 27°C ± 2°C as described earlier (56). Plants were grown under four different red light regimes. RC-plants grown for 8 weeks under intermittent red light (2 min red light every 8 h); D-plants grown as RC-plants but put into darkness after 7 weeks for 8 d; 1′R-plants grown as D-plants but treated with 1 min of red light 2 h before harvesting; FR plants grown as 1′R-plants but instead of 119 min darkness after the 1 min red light pulse, the tissue was put into far-red light for 10 min followed by darkness for 109 min. After these light treatments, the tissue was harvested under green safe lights, washed with distilled H_2O and frozen in liquid N_2.

Isolation of RNA. *Lemna* total RNA was isolated by phenol extraction and salt precipitation as previously described (19, 59). Poly(A)RNA was isolated using oligo (dT) cellulose as previously described (55).

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[3] Abbreviations: SSU, small subunit; RuBP, ribulose 1,5-bisphosphate.

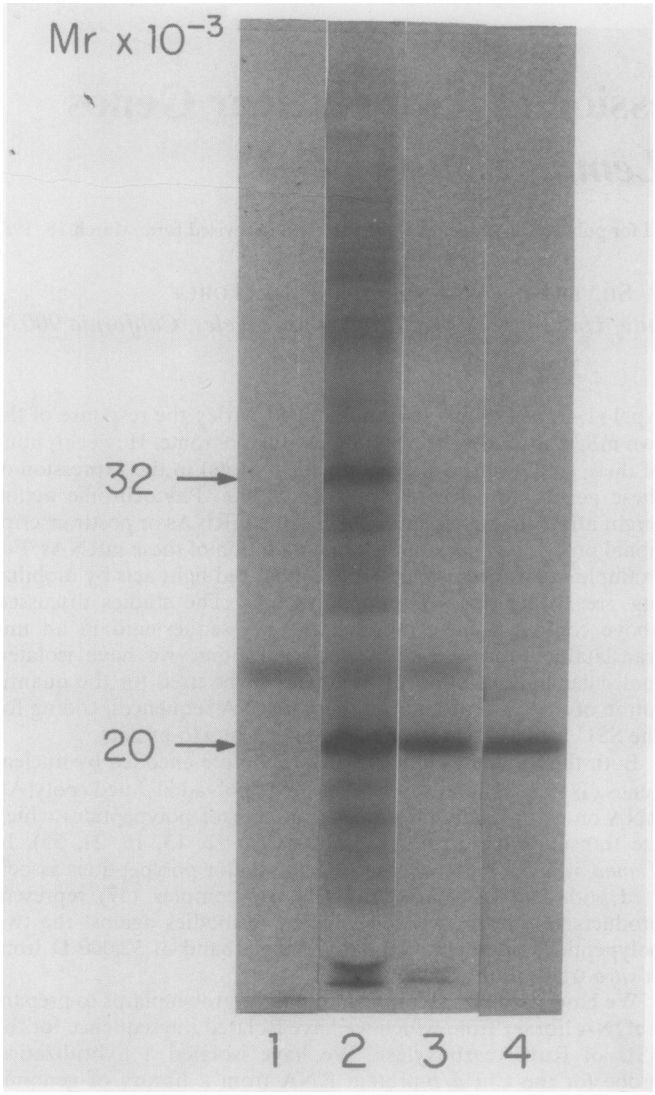

FIG. 1. Identification of a SSU clone. Fluorogram of translation products fractionated by 12.5% SDS-polyacrylamide gel electrophoresis. The numbers refer to the mol wt of the precursors to the SSU polypeptide and Chl *a/b*-protein in *L. gibba*. Lane 1, Endogenous translation products; lane 2, translation products of total poly(A)RNA from *L. gibba* grown under continuous white light; lane 3, translation products of the RNA selected by the putative SSU cDNA clone; lane 4, immunoprecipitation of the translation products produced by the selected poly(A)RNA with antibodies against the SSU polypeptide.

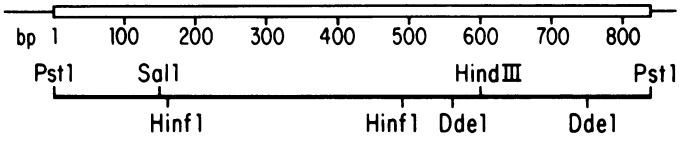

FIG. 2. Restriction endonuclease map of *L. gibba* SSU cDNA clones pLgSSU1 and pLgSSU2. The open line represents the *L. gibba* cDNA insert in the Pst 1 site of pBR322. The numbers refer to basepairs (bp).

Identification of a cDNA Clone Encoding the SSU Sequences of *L. gibba*. A more complete description of the cloning and nucleotide sequence analysis of the SSU clone will be presented elsewhere. cDNA was prepared by reverse transcription of total poly(A)RNA from *L. gibba* grown under continuous white light illumination using a procedure based on published methods (25, 40). The cDNA was converted to double-stranded DNA using the fragment A of DNA polymerase I (Bethesda Research Laboratories, Gaithersburg, MD). The hairpin loop in the double-stranded DNA was removed by S1 nuclease (44) and the DNA sized by sucrose gradient centrifugation. DNA having length of 200 basepairs and more was used for homopolymer tailing with dCTP (39). Pst 1-linearized pBR322 DNA was tailed with dGTP. Subsequently, the tailed DNAs were annealed and used to transform *Escherichia coli* strain HB101. Transformants were selected for Tet^r, Amp^s by the replica filter method (20). Such cDNA clones were arrayed on nitrocellulose filters (Schleicher & Schuell), grown, and prepared for colony hybridization (22). Differential hybridization was used to select clones containing copies of the SSU-mRNA sequence. The filters were hybridized with ^{32}P-labeled cDNA made against poly(A)RNA from both light-grown and dark-treated plants. In addition, a cDNA hybridization probe was prepared from a RNA preparation enriched for the SSU mRNA by fractionation of total poly(A)RNA on a 1% agarose/methyl-mercury-hydroxide gel. RNA was eluted from gel slices and the fraction containing the SSU mRNA was identified by translation of aliquots in a cell-free translation system from rabbit reticulocyte (34). An 11S RNA fraction codes predominantly for the SSU-polypeptide.

Plasmid DNA was isolated from the putative SSU cDNA clones by a standard high-salt procedure (15). The identity of the clones was confirmed by hybrid selection and translation of RNA from 20 μg poly(A)RNA from light-grown tissue with plasmid DNA cut with EcoR1 and bound to nitrocellulose discs (26, 36). An aliquot of the translation products was used for immunoprecipitation by antibodies against *Lemna* SSU polypeptide by the modified *Staphylococcus aureus* method (24, 28). Aliquots of the translation products and the immunoprecipitate were characterized by polyacrylamide gel electrophoresis (33) and fluorography (9).

Identification of a Bacteriophage λ Genomic Clone Encoding Chl *a/b*-Protein Sequences of *L. gibba*. A full description of the cloning and characterization of the genes for the Chl *a/b*-protein will be presented elsewhere (Wimpee *et al.*, manuscript in preparation). Briefly, *Lemna* nDNA was extracted from isolated nuclei (53), banded twice in CsCl, and subjected to a partial digestion by EcoR1 (Bethesda Research Laboratories, Inc., Gaithersburg, MD). The partially digested DNA was fractionated on 10 to 40% sucrose gradients and those fractions containing fragments between approximately 10 and 20 kbases were pooled.

λCharon 4 arms were prepared and ligated to the EcoR1 digested *Lemna* DNA (32) and packaged into recombinant phage (23).

Subsequently, the genome library was screened for clones containing the genes for the Chl *a/b*-protein (62). The first screening was done using ^{32}P-cDNA synthesized from poly(A)RNA which was highly enriched for Chl *a/b*-protein mRNA. This enrichment was accomplished as described for the SSU polypeptide mRNA (see above). A 14S RNA fraction was found to be highly enriched for Chl *a/b*-protein mRNA.

During the course of the first screening, we obtained a cDNA clone for the Chl *a/b*-protein sequence of pea from Dr. Anthony Cashmore (10). This probe was found to cross-hybridize strongly with the *Lemna* genomic clones, and was used in subsequent screening and plaque purification.

The identity of the putative Chl *a/b*-protein genomic clones was confirmed by hybrid release and translation (see above) and by immunoprecipitation using antibodies against the Chl *a/b*-protein of *Lemna* (57).

Subcloning of a 1900 Basepair Hind III Fragment from λLgAB19 into pBR322. One of the genomic clones encoding a Chl *a/b*-protein designated λLgAB19 was digested with Hind III (BRL) and the DNA fragments separated by electrophoresis on a 0.75% agarose gel. Hybridizations to DNA fragments fractionated

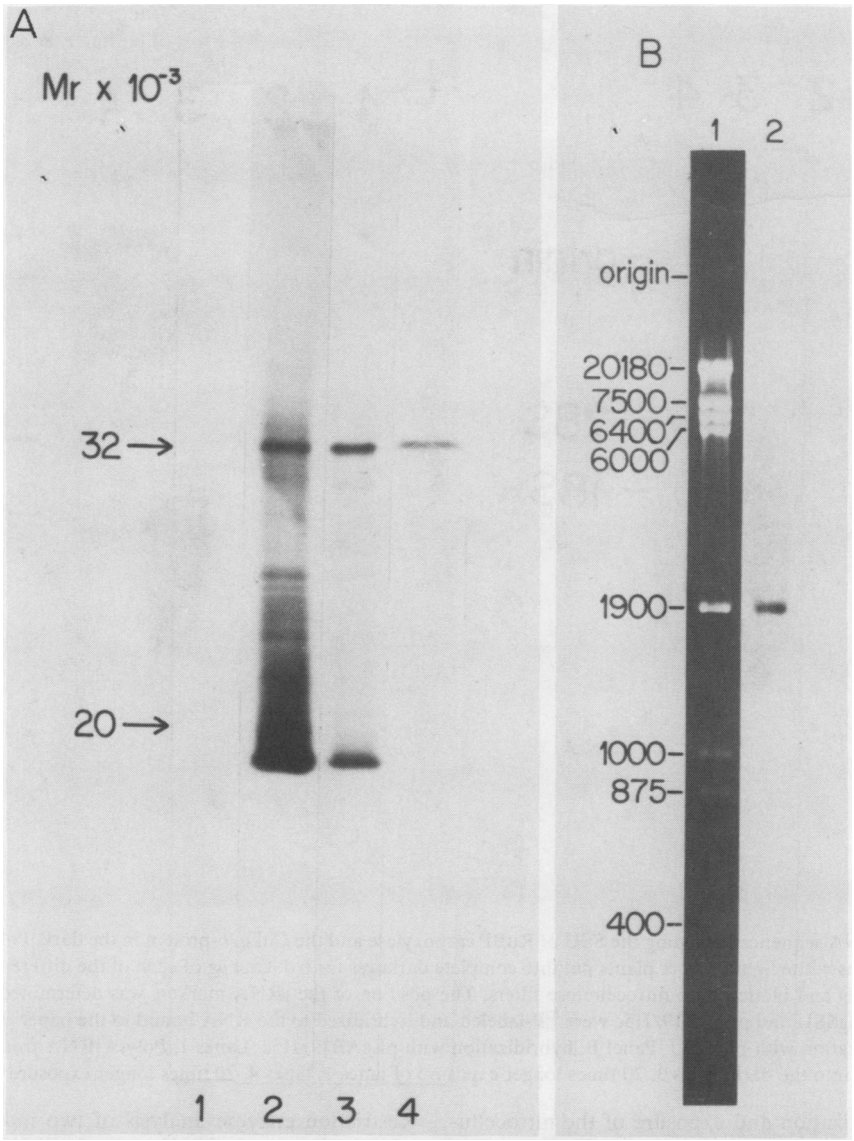

FIG. 3. Identification of a Chl *a/b*-protein clone. Panel A shows a fluorogram of translation products fractionated by SDS-polyacrylamide gel electrophoresis. The numbers refer to the mol wt of the precursors to the SSU polypeptide and the Chl *a/b*-protein in *Lemna*. Lane 1, Endogenous translation products; lane 2, translation products from total poly(A)RNA from *L. gibba* grown under continuous white light; lane 3, translation products of RNA selected by λLgAB19; lane 4, immunoprecipitation of an aliquot of translation products shown in lane 3 by antibodies against the Chl *a/b*-protein. Panel B: lane 1, a Hind III digest of λLgAB19 after electrophoresis on a 0.75% agarose gel; lane 2, an autoradiogram of the same gel is shown after hybridization *in gello* with random-primed ^{32}P-labeled cDNA, synthesized from poly(A)RNA isolated from plants grown under continuous white light.

on agarose gels were done either after blotting onto nitrocellulose (49) or in the gel itself after denaturation in 0.5 N NaOH, 1 M NaCl, neutralization in 0.5 M Tris (pH 7.5), 0.5 M NaCl, and drying under vacuum (50). We refer to the latter technique as '*in gello*' hybridization. The DNA band of interest was identified by hybridization to ^{32}P-cDNA made from *Lemna* poly(A)RNA, cut out of the agarose gel, electroeluted and ligated to Hind III digested pBR322 which had been treated with bacterial alkaline phosphatase. The ligated DNA was used to transform *E. coli* strain HB101, and transformants selected for Amp^r and Tet^s. Subsequently, plasmid DNA was isolated from selected recombinants, digested with Hind III, run on a 1% agarose gel and hybridized *in gello* with ^{32}P-cDNA, made against poly(A)RNA from white light-grown *Lemna*. A positive clone from this screening, pLgAB19/H5c, was then used as a hybridization probe in subsequent experiments.

Hybridization of Cloned Probes to *L. gibba* Total RNA and Poly(A)RNA. Total RNA or poly(A)RNA was isolated as described above. Ten μg of total RNA or 1 μg of poly(A)RNA was fractionated on 1% agarose gels in 20 mM MOPS, 1 mM EDTA, 5 mM Na-acetate, 2.2 M formaldehyde (pH 7.0), and blotted onto nitrocellulose according to Thomas (54). The position of the rRNA markers was determined by running poly(A)$^-$ RNA alongside the other tracks. Before blotting the gel this lane was cut off, stained with ethidium bromide, and photographed. To ensure that equal amounts of the RNA preparations were used, a parallel gel was run under the same conditions, stained after electrophoresis with ethidium bromide, photographed using Kodak Royal pan film (4141), and the film scanned with a Joyce Loebl densitometer.

pLgSSU or pLgAB19/H5c were labeled with ^{32}P by nick-translation (37) and hybridized to the RNA bound to the paper according to Thomas (54). However, instead of 1 times Denhardt's solution, a 10 times solution was used. The relative concentrations of the specific RNAs in Northern blots were determined by

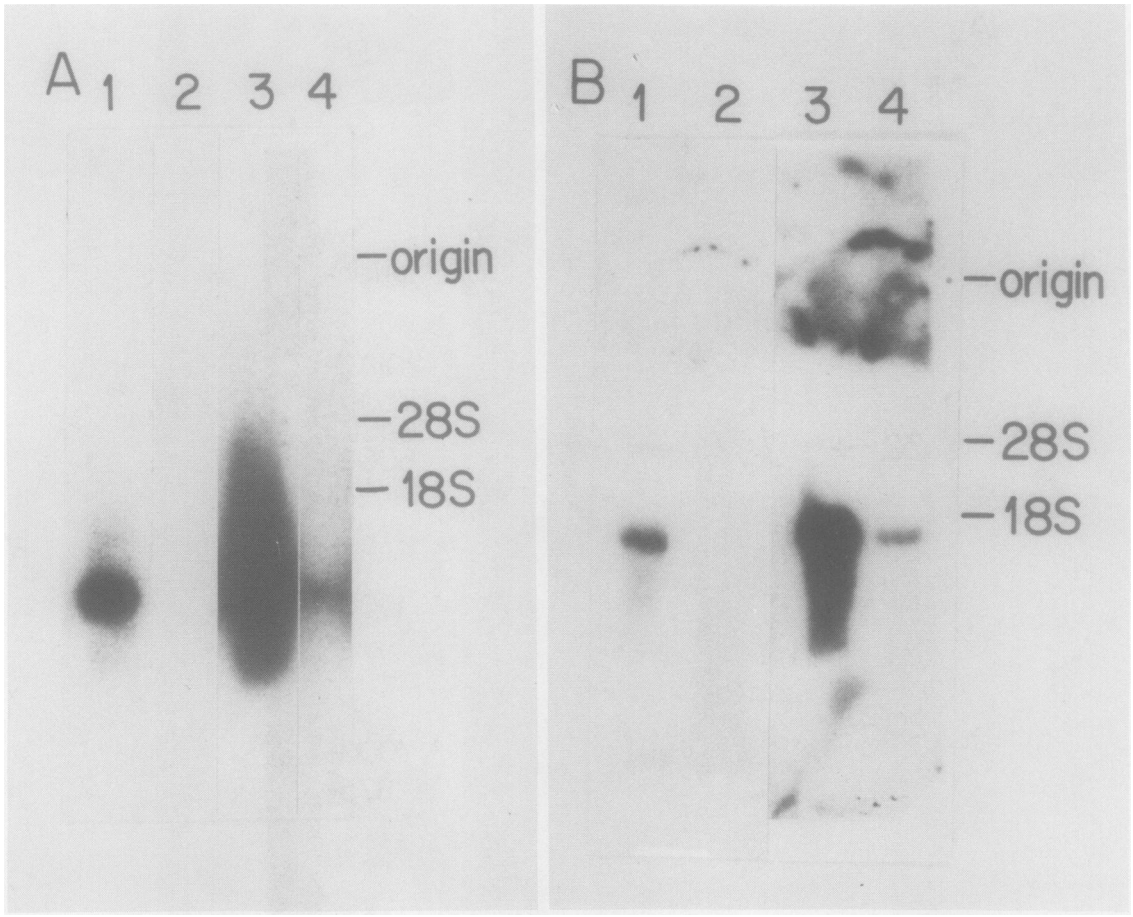

FIG. 4. Degradation of RNA sequences encoding the SSU of RuBP carboxylase and the Chl *a/b*-protein in the dark. Poly(A)RNA was isolated from plants grown under continuous white light or from plants put into complete darkness for 6 d. One μg of each of the different RNAs was fractionated on 1% agarose/formaldehyde gels and blotted onto nitrocellulose filters. The position of the rRNA markers was determined by running poly(A)⁻ RNA alongside the other tracks. pLgSSU and pLgAB19/H5c were ^{32}P-labeled and hybridized to the RNA bound to the paper. Autoradiograms of the filters are shown. Panel A, Hybridization with pLgSSU; Panel B, hybridization with pLgAB19/H5c. Lanes 1, Poly(A)RNA from light-grown tissue; lanes 2, poly(A)RNA from tissue put into the dark; lanes 3, 20 times longer exposure of lanes 1; lanes 4, 20 times longer exposure of lanes 2.

densitometry, after hybridization and exposure of the nitrocellulose filter to a preflashed x-ray film (Kodak XAR). The exposures were within the linear response range of the film (30). Subsequently, the peaks of the tracing were cut out and weighed.

RESULTS

Isolation of cDNA Clones Encoding SSU Sequences. Poly(A)RNA from *L. gibba* grown under white light for 3 weeks was used for the construction of the cDNA library. Clones coding for the SSU polypeptide were selected by a differential screening with cDNA probes made against poly(A)RNA isolated from tissue grown under different light regimes and by screening with a cDNA probe made against a RNA fraction enriched for the SSU polypeptide mRNA. The differential screening depends on the large difference in the levels of the mRNA for the SSU polypeptide present in light-grown and dark-treated plants (55, 56, 59).

The identity of cDNA clones selected this way was further established by hybrid selection and translation (36). Figure 1 demonstrates that a mRNA coding for a polypeptide with the mobility of the precursor of the SSU of RuBPCase was selected from total poly(A)mRNA by hybridization with one of the putative SSU cDNA clones. Immunoprecipitation of the translation products with an antibody against the SSU polypeptide of *Lemna* confirmed that the selected mRNA indeed codes for the precursor of this polypeptide, which has a mol wt of about 20,000 (55). Restriction enzyme analysis of two independently isolated SSU cDNA clones, pLgSSU1 and pLgSSU2, gave the physical map shown in Figure 2. Both cDNA clones possess the same restriction map and contain an insert length of about 850 basepairs. Sequence analysis (Stiekema, Wimpee, and Tobin, manuscript in preparation) has confirmed that the clone contains the SSU coding sequence.

Isolation of Genomic Clones Encoding Chl *a/b*-Protein Sequences. The library of *Lemna* genomic DNA was screened using ^{32}P-labeled cDNA synthesized from poly(A)RNA which had been enriched for the mRNA for the Chl *a/b*-protein by fractionation on a methyl-mercury hydroxide/agarose gel. Positive clones from this screening were plaque purified using a cDNA clone for the Chl *a/b*-protein sequence from pea, which was given to us by Dr. A. Cashmore, The Rockefeller University, New York (10). A genomic clone designated λLgAB19 was identified as containing the coding sequence for the Chl *a/b*-protein by hybrid selection and translation in the *in vitro* system from wheat germ (38) (Fig. 3A). Immunoprecipitation of the translation products with an antibody against the Chl *a/b*-protein (60) showed that the selected mRNA indeed codes for the 32,000 D precursor of the Chl *a/b*-protein (58). Restriction enzyme mapping of λLgAB19 showed that it contains a 15.8 kbasepair insert.

Subcloning of a Chl *a/b*-Protein Sequence. The coding sequence contained in a 1900 basepair Hind III fragment of λLgAB19 was subcloned into pBR322 so that we could prepare a high-specific-

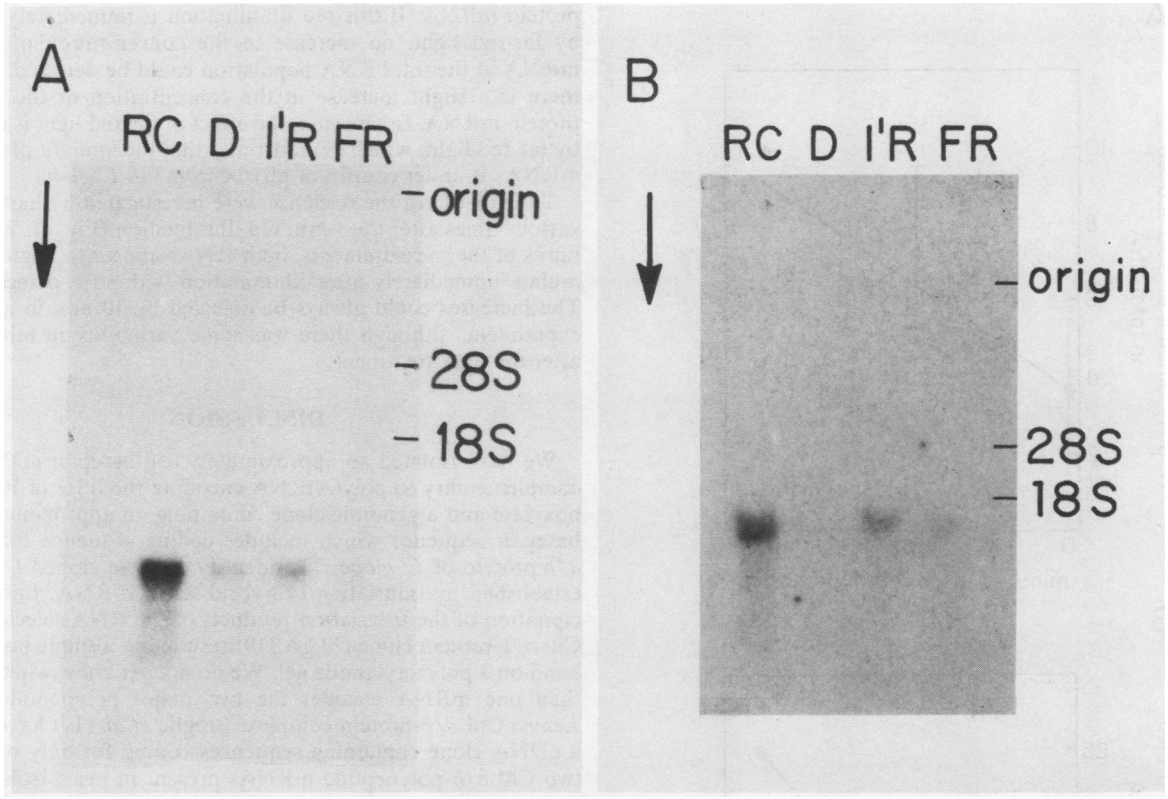

FIG. 5. Phytochrome regulation of the RNA sequences encoding the SSU of RuBP carboxylase and the Chl *a/b*-protein. Plants were grown under four different light regimes: RC, D, 1'R, FR. After harvest, total RNA of each sample was isolated. Ten μg of each RNA sample was run on a 1% agarose/formaldehyde gel, blotted onto nitrocellulose, and subsequently hybridized to pLgSSU (panel A) and pLgAB19/H5c (panel B), ^{32}P-labeled by nick-translation. The photographs show autoradiograms of the filters. RC, D, 1'R, and FR symbols refer to the light regime used.

activity hybridization probe for the Chl *a/b*-protein. Figure 3B shows a Hind III digest of λLgAB19, after electrophoresis on a 0.75% agarose gel. The second lane is an autoradiogram of the same gel after hybridization *in gello* with random-primed ^{32}P-labeled cDNA, synthesized from poly(A)RNA isolated from plants grown under continuous white light. This experiment demonstrates that a fragment of 1900 basepairs contains the coding sequence. This band was subcloned in pBR322. The 1900 basepair band is actually a doublet, one fragment of which contains the Chl *a/b*-protein coding sequence. Therefore, plasmid DNA was isolated from the selected recombinants, digested with Hind III, run on a 1% agarose gel, and hybridized with ^{32}P-cDNA made against poly(A)RNA from white-light-grown *Lemna* (data not shown). A positive clone from this screening, designated pLgAB19/H5c, was then used as a probe in subsequent experiments. This subclone also hybridizes to the pea Chl *a/b*-protein clone.

Degradation of the mRNAs for the SSU Polypeptide and the Chl *a/b*-Protein in the Dark. In *Lemna* the amounts of translatable mRNAs for the SSU of RuBP carboxylase and the Chl *a/b*-protein relative to other translatable mRNAs decrease when plants are put into the dark (55, 56, 59). The decline is not due to dilution during growth because in this species growth in the dark is so slowed in the absence of intermittent illumination that it is not significant during a 1-week dark period. We have used the cloned probes to demonstrate that this decline must involve degradation of the mRNA rather than a conversion to an untranslatable form (Fig. 4). ^{32}P-labeled pLgSSU (A) and pLgAB19/H5c (B) were hybridized to poly(A)RNA isolated from plants grown in white light (lanes 1 and 3) or put into the dark for 7 d (lanes 2 and 4). Figure 4A also shows that pLgSSU hybridized to a RNA species which has a length of about 900 nucleotides in the sample from plants grown under continuous illumination. This is in accordance with the length reported for pea SSU-mRNA (6, 10). A RNA species with the same mobility as found in the light-grown tissue could be detected in the sample from dark-treated plants only after long exposure of the filter (lane A4).

Figure 4B shows that pLgAB/H5c hybridizes to a RNA species of about 1100 nucleotides in the sample from plants grown under continuous white light illumination. This length for the Chl *a/b*-protein mRNA is in accordance with the approximate length reported by Broglie *et al.* for this mRNA in pea (10). Again, a RNA species of the same mobility as found in the light-grown tissue could be detected in the sample from dark-treated plants only after long exposure of the filter (lane B4).

Role of Phytochrome in the Control of the Expression of the SSU Polypeptide and Chl *a/b*-Protein Genes. To investigate the role of phytochrome in the control of the expression of these two genes, we compared the amount of each mRNA present in *L. gibba* grown under different light regimes. We isolated total RNA from plants grown in the dark with 1 min of red light every 8 h for 8 weeks (RC-plants). Plants were also grown under the same regime but only for 7 weeks, and then put into complete darkness for 8 d before harvesting (D-plants). In addition, dark-treated plants were illuminated with either 1 min red light (R-plants) or 1 min red light followed immediately by 10 min far-red light (FR-plants) 2 h before harvesting. Equal amounts of these four different preparations of total RNA were run on a 1% agarose-formaldehyde gel and subsequently blotted onto nitrocellulose. The relative concentration of the SSU and Chl *a/b*-protein mRNA in each of the four RNA preparations was determined by hybridization of the filter with pLgSSU and pLgAB19/H5c, ^{32}P-labeled by nick-translation (Fig. 5). This experiment demonstrates that phytochrome action can substantially change the concentration of

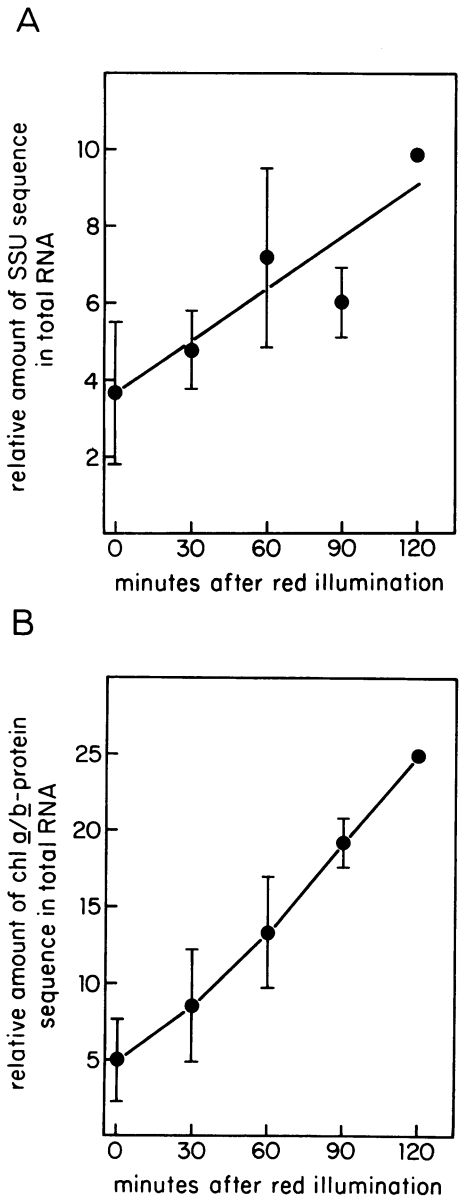

FIG. 6. Kinetics of the response to red illumination. Dark-treated plants (D plants) were given 1 min red illumination and then harvested at the times indicated. RNA was isolated and the samples treated as described for Figure 5. Twenty μg of total RNA was used per lane. The relative concentrations of the hybridizable sequences were determined by densitometry of autoradiograms. The total RNA concentration of each sample was also determined by densitometry as described in materials and methods. A, Red light-induced increase in the SSU sequence; B, Red light-induced increase in the Chl *a/b*-protein sequence. The results presented are an average of four (for SSU) or three (for Chl *a/b*-protein) separate hybridization experiments normalized to the same relative values for the 120-min sample. The SD for the other samples are indicated.

and SSU and Chl *a/b*-protein sequences within 2 h. Plants grown in the dark with 1 min of red light every 8 h contain a considerable amount of both mRNAs. When these plants are put into the dark for 8 d, the relative concentration of the SSU mRNAs drops about 8-fold, while the relative Chl *a/b*-protein mRNA concentration drops to a hardly detectable level as measured by this method. However, 1 min of red light 2 h before harvesting of the dark tissue resulted in a 2- to 3-fold increase in the amount of SSU mRNA and an even larger increase in the amount of Chl *a/b*-protein mRNA. If this red illumination is immediately followed by far-red light, no increase in the concentration of the SSU mRNA in the total RNA population could be detected, although there is a slight increase in the concentration of the Chl *a/b*-protein mRNA. In any case, the effect of the red light is reversible by far-red light, which demonstrates that the amount of these two mRNAs is under control of phytochrome in *L. gibba*.

The kinetics of the response were investigated by harvesting at various times after the 1 min red illumination (Fig. 6). Within the limits of the measurements, both RNAs appear to begin to accumulate immediately after illumination without a detectable lag. The increases could always be detected by 30 min in any single experiment, although there was some variability in initial levels after the dark treatment.

DISCUSSION

We have isolated an approximately 850 basepair cDNA clone complementary to poly(A)RNA encoding the SSU of RuBP carboxylase and a genomic clone containing an approximately 1900 basepair sequence which includes coding sequence for the Chl *a/b*-protein of *L. gibba*. The identity of these cloned DNAs was established by translation of hybrid-selected RNA. Immunoprecipitation of the translation products of the RNA selected by the Chl *a/b*-protein clone (λLgAB19) resulted in a single polypeptide band on a polyacrylamide gel. We do not yet know whether more than one mRNA encodes the two major polypeptides of the *Lemna* Chl *a/b*-protein complex. Broglie *et al.* (10) have isolated a cDNA clone containing sequences coding for only one of the two Chl *a/b*-polypeptide mRNAs present in peas. Isolation and characterization of other genomic clones coding for the Chl *a/b*-protein present in the *Lemna* genomic library will clarify the situation in *Lemna*.

We have used the probes for the two specific RNAs to investigate how the effect of light on gene expression in *Lemna* is mediated. We have compared the relative concentration of each RNA present in plants grown under different light regimes. Plants grown in the dark with 2 min red light every 8 h contain high levels of the RNAs for the SSU and the Chl *a/b*-protein. These RNA concentrations decline rapidly when plants are put into complete darkness. The observation that a low amount of the SSU sequence is present after dark treatment of the plants grown with intermittent red light is in agreement with results based on *in vitro* translation of *Lemna* RNA (56), but it differs from the results of Smith and Ellis (48) who could not detect any SSU mRNA in dark-grown peas. It is difficult to compare these results directly because Smith and Ellis used DBM-paper for transfer of the RNA, and this method is less sensitive than nitrocellulose (54). It is possible that there is a species difference or that the low level detected in *Lemna* is not due to synthesis of the sequence in the dark, but is mRNA remaining from the illuminated plants. Tobin and Turkaly (60) found that kinetin can retard the degradation of this mRNA. Berry-Lowe *et al.* (8) have found low levels of the SSU sequence in dark-grown soybeans.

Illumination of dark-treated plants with red light can cause increases in the concentrations of both RNAs within 30 min (Fig. 6). The effect of red light can be reversed by 10 min of far-red light immediately after the 1 min red light pulse, and thus the response is mediated by phytochrome. The phytochrome response is very rapid and does not appear to have a substantial lag time. The increase in the SSU sequence in response to red in *Lemna* is much faster than that seen in dark-grown peas illuminated with white light where there may be a lag period (45). However, in barley, changes in levels of translatable mRNA occur rapidly in response to phytochrome action (1). Extremely rapid increases in specific RNA sequences have also been reported for a number of heat-shock proteins in soybean (42).

The presence of RNA sequences for a certain protein does not

necessarily mean that the mRNA is also available for translation (*cf.* 18, 61). Therefore, we reconfirmed our earlier results by translating total RNA isolated from tissue grown under the different light regimes used in this study in an *in vitro* translation system from rabbit reticulocytes. The polypeptide amounts corresponding to the two mRNAs matched our Northern hybridization data (data not shown). This experiment confirms that both mRNAs are available for translation.

However, *in vivo* the synthesis of the Chl *a/b*-protein occurs only at a very low level in plants grown under intermittent red light (47, 56) and the protein does not accumulate in the thylakoid membranes (7, 56). In contrast, the SSU polypeptide is synthesized under this light regime. The possibility that the mRNA for the Chl *a/b*-protein is present in an untranslatable form is ruled out by the *in vitro* translation experiments. The mRNA is associated with polysomes (47), so it is not likely that light is affecting mobilization of the mRNA or the processing of the transcript to a translatable form. Apel (1) has suggested that the synthesis and accumulation of this protein depends on the presence of Chl, and Apel and Kloppstech (4) and Cuming and Bennett (14) have presented evidence for a breakdown of newly synthesized apoprotein in the absence of Chl *a* and *b*. The results presented here and by Slovin and Tobin (47) are consistent with these hypotheses. In addition, indirect evidence suggests that there is an additional light-dependent process necessary for the translation of the Chl *a/b*-protein mRNA (47).

In summary, the results reported here demonstrate that phytochrome can rapidly affect the expression of the genes for the small subunit of RuBP carboxylase and Chl *a/b*-protein in *L. gibba*. Red light can induce rapid and specific increases in the amounts of the RNA encoding these polypeptides. Therefore, phytochrome action must change either the transcription rate or rate of degradation of these mRNAs. The exact mechanism of phytochrome action, however, remains unknown. Because phytochrome is found primarily in the cytoplasm and has been reported to be associated with all cellular compartments and not only the nucleus, it is unlikely that the phytochrome molecule itself acts directly on the genes to stimulate their transcription. A more likely hypothesis might involve another factor such as has been described for prolactin (51). We anticipate that studies with isolated nuclei from *Lemna* will enable us to clarify the way in which phytochrome can affect gene expression.

LITERATURE CITED

1. Apel K 1979 Phytochrome-induced appearance of mRNA activity for the apoprotein of the light-harvesting chlorophyll a/b-protein of barley (*Hordeum vulgare*). Eur J Biochem 97: 183–188
2. Apel K 1981 The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). Phytochrome-induced decrease of translatable mRNA coding for the NADPH: protochlorophyllide oxidoreductase. Eur J Biochem 120: 89–93
3. Apel K, K Kloppstech 1978 The plastid membranes of barley (*Hordeum vulgare*). Light-induced appearance of mRNA coding for the apoprotein of the light-harvesting chlorophyll a/b-protein. Eur J Biochem 85: 581–588
4. Apel K, K Kloppstech 1980 The effect of light on the biosynthesis of the light-harvesting chlorophyll a/b-protein. Evidence for the requirement of chlorophyll a for the stabilization of the apoprotein. Planta 150: 426–430
5. Bedbrook JR, G Link, DM Coen, L Bogorad, A Rich 1978 Maize plastid gene expressed during photoregulated development. Proc Natl Acad Sci USA 75: 3060–3064
6. Bedbrook JR, SM Smith, RJ Ellis 1980 Molecular cloning and sequencing of cDNA encoding the precursor to the small subunit of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase. Nature (Lond) 287: 692–697
7. Bennett J 1981 Biosynthesis of the light-harvesting chlorophyll a/b-protein. Polypeptide turnover in darkness. Eur J Biochem 118: 61–70
8. Berry-Lowe SL, TD McKnight, DM Shah, RB Meagher 1982 The nucleotide sequence, expression, and evolution of one member of a multigene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybeans. J Mol Appl Genet 1: 483–498
9. Bonner WM, RA Laskey 1974 A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur J Biochem 46: 83–88
10. Broglie R, G Bellemare, SG Bartlett, N-H Chua, AR Cashmore 1981 Cloned DNA sequences complementary to mRNAs encoding precursors to the small subunit of ribulose-1,5-bisphosphate carboxylase and a chlorophyll a/b binding polypeptide. Proc Natl Acad Sci USA 78: 7304–7308
11. Cashmore AR 1979 Reiteration frequency of the gene coding for the small subunit of ribulose-1,5-bisphosphate carboxylase. Cell 17: 383–388
12. Cashmore AR, MK Broadhurst, RE Gray 1978 Cell-free synthesis of leaf protein: identification of an apparent precursor of the small subunit of ribulose-1,5-bisphosphate carboxylase. Proc Natl Acad Sci USA 75: 655–659
13. Chua N-H, GW Schmidt 1978 Transport of proteins into mitochondria and chloroplasts. Proc Natl Acad Sci USA 75: 6110–6114
14. Cuming AC, J Bennett 1981 Biosynthesis of the light-harvesting chlorophyll a/b-protein. Control of messenger RNA activity by light. Eur J Biochem 118: 71–80
15. Davis RW, D Botstein, JR Roth (1980) Advanced Bacterial Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 116–125
16. Dobberstein B, G Blobel, N-H Chua 1977 *In vitro* synthesis and processing of a putative precursor for the small subunit of ribulose-1,5-bisphosphate carboxylase of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 74: 1082–1085
17. Ellis RJ 1979 The most abundant protein in the world. Trends Biochem Sci 4: 241–244
18. Giles AB, D Grierson, H Smith 1977 *In vitro* translation of messenger-RNA from developing bean leaves. Evidence for the existence of stored messenger RNA and its light-induced mobilization into polyribosomes. Planta 136: 31–36
19. Hafner MH, MB Chin, BG Lane 1978 Wheat embryo ribonucleates XII. Formal characterization of terminal and penultimate nucleoside residues at the 5′-ends of 'capped' RNA from imbibing wheat embryos. Can J Biochem 56: 729–733
20. Hanahan D, M Meselson 1980 Plasmid screening at high colony density. Gene 10: 63–67
21. Highfield PE, RJ Ellis 1978 Synthesis and transport of the small subunit of chloroplast ribulose bisphosphate carboxylase. Nature (Lond) 271: 420–424
22. Hoeijmakers JHJ, P Borst, J van den Burg, C Weissman, GAM Cross 1980 The isolation of plasmids containing DNA complementary to messenger RNA for variant surface glycoproteins of *Trypanosoma brucei*. Gene 8: 391–417
23. Hohn B 1979 *In vitro* packaging of λ and cosmid DNA. Methods Enzymol 68: 299–309
24. Ivarie RD, PP Jones 1979 A rapid sensitive assay for specific protein synthesis in cells and in cell free translations: use of *Staphylococcus aureus* as an adsorbent for immune complexes. Anal Biochem 97: 24–35
25. Kacian DL, JC Myers 1976 Synthesis of extensive, possible complete, DNA copies of polio virus RNA in high yields and at high specific activities. Proc Natl Acad Sci USA 73: 2191–2195
26. Kafatos FC, CW Jones, A Efstratiadis 1979 Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res 7: 1541–1552
27. Kawashima N, SG Wildman 1972 Studies on fraction I protein IV. Mode of inheritance of primary structure in relation to whether chloroplast or nuclear DNA contains the code for a chloroplast protein. Biochim Biophys Acta 262: 42–49
28. Kessler SW 1975 Rapid isolation of antigens from cells with a staphycoccyl protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J Immunol 115: 1617–1624
29. Kung SD, JP Thornber, SG Wildman 1972 Nuclear DNA codes for the photosystem II chlorophyll-protein of chloroplast membranes. FEBS Lett 24: 185–188
30. Laskey RA, AD Mills 1975 Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur J Biochem 56: 335–341
31. Link G 1982 Phytochrome control of plastid mRNA in mustard (*Sinapis alba* L.) Planta 154: 81–86
32. Maniatis T, RC Hardison, E Lacy, J Lauer, C O'Connell, D Quon, GK Sim, A Efstratiadis 1978 The isolation of structural genes from libraries of eucaryotic DNA. Cell 15: 687–701
33. O'Farrell PH 1975 High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021
34. Pelham HRB, RJ Jackson 1976 An efficient mRNA-dependent translation system from reticulocyte lysates. Eur J Biochem 67: 247–256
35. Reisfeld A, J Gressel, KM Jakob, M Edelman 1978 Characterization of the 32,000 dalton membrane protein-I. Early synthesis during photoinduced plastid development of *Spirodela*. Photochem Photobiol 27: 161–165
36. Ricciardi RP, JS Miller, BE Roberts 1979 Purification and mapping of specific mRNAs by hybridization-selection and cell-free translation. Proc Natl Acad Sci USA 76: 4927–4931
37. Rigby PWJ, M Dieckmann, C Rhodes, P Berg 1977 Labeling deoxyribunucleic acid to high specific activity *in vitro* by nick-translation with DNA polymerase I. J Mol Biol 113: 237–251
38. Roberts BE, BM Paterson 1973 Efficient translation of tobacco mosaic virus RNA and rabbit globin 9s RNA in a cell-free system from commercial wheat germ. Proc Natl Acad Sci USA 70: 2330–2334
39. Roychoudhury R, E Jay, R Wu 1976 Terminal labelling and addition of homopolymer tracts to duplex DNA fragments by terminal deoxynucleotidyl transferase. Nucleic Acids Res 3: 101–116
40. Salser W 1978 Cloning cDNA sequences: a general technique for propagating eukaryotic gene sequences in bacterial cells. *In* AM Chakrabarty, ed, Genetic Engineering. CRC Press, Cleveland, OH, pp 53–81
41. Santel H-J, K Apel 1981 The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). The effect of light on the NADPH: protochlorophyllide

oxido reductase. Eur J Biochem 120: 95–103
42. SCHÖFFL F, J KEY 1982 An analysis of mRNAs for a group of heat shock proteins of soybeans using cloned DNAs. J Mol Appl Genet 1: 301–314
43. SCHRÖDER J, F KREUZALER, E SCHÄFER, K HAHLBROCK 1979 Concomitant induction of phenylalanine ammonia-lyase and flavanone synthase mRNAs in irradiated plant cells. J Biol Chem 254: 57–65
44. SHENK TE, C RHODES, P RIGBY, P BERG 1975 Biochemical method for mapping mutational alterations in DNA with S_1 nuclease: the location of deletions and temperature-sensitive mutations in simian virus 40. Proc Natl Acad Sci USA 72: 989–992
45. SHINOZAKI K, Y SASAKI, T SAKIHAMA, T KAMIKUBO 1982 Coordinate light-induction of two mRNAs, encoded in nuclei and chloroplasts, of ribulose-1,5-bisphosphate carboxylase oxygenase. FEBS Lett 144: 73–76
46. SIMS TL, DR HAGUE 1981 Light-stimulated increase of translatable mRNA for phosphoenolpyruvate carboxylase in leaves of maize. J Biol Chem 256: 8252–8255
47. SLOVIN JP, EM TOBIN 1982 Synthesis and turnover of the light-harvesting chlorophyll a/b-protein in *Lemma gibba* grown with intermittent red light: possible translational control. Planta 154: 465–472
48. SMITH SM, RJ ELLIS 1981 Light stimulated accumulation of transcripts of nuclear and chloroplast genes for ribulosebisphosphate carboxylase. J Mol Appl Genet 1: 127–137
49. SOUTHERN EM 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–517
50. STIEGLER GL, HM MATTHEWS, SE BINGHAM, RB HALLICK 1982 The gene for the large subunit of ribulose-1,5-bisphosphate carboxylase in *Euglena gracilis* chloroplast DNA: locations, polarity, cloning, and evidence for an intervening sequence. Nucleic Acids Res 10: 3427–3444
51. TEYSSOT B, L-M HOUDEBINE, J DJIANE 1981 Prolactin induces release of a factor from membranes capable of stimulating β-casein gene transcription in isolated mammary cell nuclei. Proc Natl Acad Sci USA 78: 6729–6733
52. THORNBER JP 1975 Chlorophyll-proteins: light-harvesting and reaction center components of plants. Annu Rev Plant Physiol 26: 127–158
53. TIMBERLAKE W 1978 Low repetitive DNA content in *Aspergillus nidulans*. Science 202: 973–975
54. THOMAS PS 1980 Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5205
55. TOBIN EM 1978 Light regulation of specific mRNA species in *Lemna gibba* L. G-3. Proc Natl Acad Sci USA 75: 4749–4753
56. TOBIN EM 1981 Phytochrome mediated regulation of messenger RNAs for the small subunit of ribulose-1,5-bisphosphate carboxylase and the light-harvesting chlorophyll a/b-protein in *Lemna gibba*. Plant Mol Biol 1: 35–51
57. TOBIN EM 1981 Light regulation of the synthesis of two major nuclear-coded chloroplast polypeptides in *Lemna gibba*. *In* G Akoyunoglou, ed, Photosynthesis V. Chloroplast Development. Balaban International Science Services, Philadelphia, pp 949–959
58. TOBIN EM 1981 White light effects on the mRNA for the light-harvesting chlorophyll *a/b*-protein in *Lemna gibba*. L. G-3. Plant Physiol 67: 1078–1083
59. TOBIN EM, JL SUTTIE 1980 Light effects on the synthesis of ribulose-1,5-bisphosphate carboxylase in *Lemna gibba*. L. G-3. Plant Physiol 65: 641–647
60. TOBIN EM, E TURKALY 1982 Kinetin affects rates of degradation of mRNAs encoding two major chloroplast proteins in *Lemna gibba*. L. G-3. J Plant Growth Regul 1: 3–13
61. VAN EE J 1981 Biogenesis of chloroplasts in *Spirodela oligorrhiza*. PhD Thesis. Vrije Universiteit, Amsterdam
62. WOO SLC 1979 A sensitive and rapid method for recombinant phage screening. Methods Enzymol 68: 389–395